REMARKS

I. Status of the Claims

Claims 1-43 are pending in the application. Claims 30-43 stand withdrawn pursuant to a restriction requirement and are hereby canceled.

II. Obviousness-Type Double-Patenting

A. Serial No. 09/880,609

Claim 29 is provisionally rejected under the judicially-created doctrine of obviousness-type double-patenting over claims 31 and 43 of copending application Serial No. 09/880,609. Applicants respectfully traverse.

The examiner entered a restriction requirement in the present application, finding two groups of claims representing ***patentably distinct inventions*** – claims 1-29 and claims 30-43. Applicants elected to prosecute claims 1-29, and filed a divisional application on claims 30-43, which is the '609 application. Because the examiner restricted these groups of claim, thereby acknowledging their separate patentability, it is improper to now indicate that the claims are ***not*** separately patentable:

> 35 U.S.C. 121 authorizes the Commission to restrict the claims in a patent application to a single invention when independent and distinct inventions are presented for examination. ***The third sentence of 35 U.S.C. 121 prohibits the use of a patent issuing on an application with respect to which a requirement for restriction has been made, or on an application filed as a result of such a requirement, as a reference against any divisional application.***

MPEP §804.01 (emphasis added). That is precisely what the examiner has done here. Reconsideration and withdrawal of this rejection is respectfully requested.

Also, the examiner provisionally rejects claims 30-43 of the present application over the same claims of the '609 application. As claims 30-43 are canceled herein, the rejection is rendered moot. Therefore, reconsideration and withdrawal of this rejection also is respectfully requested.

B. U.S. Patent 6,194,191

Claims 1-29 are rejected over claims 1-5, 9, 11-18, 20, 21, 30-39, 41-47, 50-53, 61-64, 66-69, 71-74, 78-80 and 86-89 of U.S. Patent 6,194,191. According to the examiner, the methods are not distinct since the recitation in the present regarding infection at a particular time would not distinguish the '191 patent since "a cell culture comprises cells in all phases of the [cell] cycle." Applicants traverse.

The examiner has misconstrued the recitation of step (b) in the present claims, which states "infecting producer cells in the culture with recombinant adenovirus, wherein the producer cells are infected between mid-log phase of growth and stationary phase of growth." This issue also bears directly on the §102 and §103 rejections, discussed below.

The discussion of "mid-log" and "stationary" phases does not relate to phases of the cell cycle. In fact, applicants are unaware of the use of these terms with regard to cell cycle stages. Cell cycle phases are M (mitotic), G_1 (gap), S (synthesis) and G_2 (post-S, pre-M).[1] Rather, "mid-log" and "stationary" phases relate to the state of the culture population, *i.e.*, where in the population growth curve the culture should be infected. In this regard, applicants direct the examiner's attention to the specification at pages 20-22, where this topic is explored:

[1] Molecular Biology of the Cell, Alberts *et al.*, eds., Garland Publishing, NY, 1983, pp. 611-612 (Exhibit 2).

Claim 3: The examiner has questioned the meaning of the term "homogenous." The examiner has interpreted this claim elsewhere as dealing with the same cell type and cell cycle phase. As explained, the present invention does not address cell cycle phase, but rather, population growth phase. With that correction, the examiner's interpretation is correct. Applicants have provided an amendment to clarify this point. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 4: The examiner argues that the term "at least a portion of the time" is unclear. Applicants traverse. The complete claims states "wherein the producer cells are perfused for at least a portion of the time ***that the cells are cultured***." This clearly specifies the time at which the perfusion may take place. So, this can clearly cover steps (a), (b), or both, and the claims are definite on this point. Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 20: The examiner states that claim 20 lacks antecedent basis for the term "producer cells." Applicants point out that claim 20 depends on claim 19, which in turn depends on claim 18, which in turn depends on claim 1. Claim 1 recites "producer cells." It is black letter law that a dependent claim carries over all of the limitations of claims from which it depends. Thus, claim 1 provides antecedent basis for the term "producer cells." Reconsideration and withdrawal of the rejection is respectfully requested.

Claim 23: The examiner argues that the term "recombinant gene" is not supported. Amendments have been provided to address this issue. Reconsideration and withdrawal of the rejection is requested.

Claim 25: The examiner argues that the term "said promoter" is not supported. Amendments have been provided to address this issue. Reconsideration and withdrawal of the rejection is requested.

B. First Paragraph

Claims 26-28 are rejected under the first paragraph of §112 as lacking a written description of the claimed invention. According to the examiner, the specification "does not reasonably support the specific exclusion of freeze-thaw method." More particular, it is stated that "although the specification ***reasonably*** conveys a variety of methods for lysing cells, the specification is not seen as reasonably conveying the concept of "any method except freeze-thaw" (emphasis added). Applicants respectfully traverse.

It is black letter law that original claims provide their own support. "Original claims constitute their own description." *In re Koller*, 613 F.2d 819, 204 U.S.P.Q. 702 (CCPA 1980), citing *In re Gardner*, 475 F.2d 389, 177 U.S.P.Q. 396 (CCPA 1973) ("Claim 2, which apparently was an original claim, in itself constituted a description in the original disclosure equivalent in scope and identical in language to the total subject matter now being claimed. See *In re Anderson*, 471 F.2d 1237, 176 U.S.P.Q. 331 (CCPA 1973). Nothing more is necessary for compliance with the description requirement of the first paragraph of 35 U.S.C. 112."). This is especially true where "a representative number of species" are adequately described in the specification, and they are representative of the entire genus. MPEP §2163 (a)(ii). Here, the specification sets for practically all the known methods for lysing cells (pages 37-44), and applicants merely have excluded one method from their claims. See also *LNP Engineering Plastic, Inc. v. Kawasaki Chemical Holding Co.* (Fed. Cir., Dec. 28, 2001).

respectfully submit that the examiner has not carried the PTO's burden in establishing that the reference teaches all the limitations of the present claims. *In re Marshall*, 198 USPQ 344 (CCPA 1978) ("... [T]o constitute anticipation, all material elements recited in a claim must be found in one unit of the prior art.").

Assuming the examiner had shifted the burden to applicants, which applicants dispute, the following observations are submitted, and are supported in the accompanying declaration of Dr. Gallagher. As discussed above, Huyghe *et al.* mentions infection of cells at 2-2.5 days, and at a confluency of 50-60%. However, also as noted above, seeding density, lag phase and doubling time of the cells under their care are not provided. Thus, one can only try to extrapolate from other sources to estimate what "phase" this population was in when infected at 2-2.5 days and 50-60% confluency. Gallagher Declaration, ¶7.

If one assumes that Huyghe *et al.* seeded at a seeding density of approximately 1-3 x 10^4 cells/cm^2, and that the 293 cells employed by Huyghe *et al.* had a lag phase of approximately 24 hours and a doubling time of approximately 36 hours, then one can calculate the phase at 50-60% confluency as early log phase, certainly less than mid-log phase, using the following calculations:

- **Initial density (midpoint of assumption range) = 2 x 10^4 cells/cm^2**
- **Growth period (2.5 days – lag time) = 1.5 days = 36 hours**
- **With a doubling time of 36 hours, the cells population will double once, giving final concentration equal to 2 x 10^4 cells/cm^2 x 2 = 4 x 10^4 cells/cm^2, consistent with early log phase density**

Gallagher Declaration at ¶7.

There are a number of scientific publications that support these conclusions. First, the examiner is directed to to Freshney, R.I., "Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed." [Exhibit 3]. At page 240, it is stated that "[t]oward the end of the log phase, the culture becomes confluent—*i.e.*, all the available growth surface is occupied and all the cells are in contact with surrounding cells." Based on this statement, it seems unlikely that Huyghe *et al.*'s cells, at 50-60% confluency, would be in late log phase.

In MediaTech's Technical Information bulletin [Exhibit 4], it is indicated, in the first paragraph of column 2 on the first page, that in order to ensure that cultures are in log phase, they must be at least 70% confluent. Thus, a culture that is only 50-60% confluent like that of Huyghe *et al.* is likely not in log phase, or at the very least, only in early log phase, not mid-log or late-log phase.

Finally, the article of Kuchler, "Biochemical methods in Cell Culture and Virology" [Exhibit 5] is relied upon. At page 90, the lag phase, which precedes the log phase, is said to vary from 24 to 48 hours. Given that Huyghe *et al.* infected cells between 48-60 hours after seeding, Kuchler suggests that Huyghe *et al.*'s cells might be barely out of lag phase, if at all. While specific cell lines or strains of cell lines can demonstrate significant variation in characteristic doubling times and duration of lag phase, Dr. Gallagher's own experience with 293 cells from various sources indicates that lag times of 24-48 hours are not uncommon after passaging.

Thus, Dr. Gallagher concludes that:

> Although none of the preceding evidence presents conclusive proof as to the precise point in the population growth curve at which Huyghe *et al.* infected cells, it is my opinion, based on the available evidence and my sample calculation, that the cells infected by Huyghe *et al.* were likely, at the very latest, in early log

phase, and not within the "mid-log phase to stationary growth phase" as specified by the pending claims.

In light of the preceding explanation, applicants respectfully submit that, as a matter of law, Huyghe cannot anticipate the instant claims as it fails to teach each element thereof. In addition, the factual evidence now of record strongly suggests that Huyghe *et al.* does not anticipate the present invention. Reconsideration and withdrawal of the rejection is requested.

V. Rejections Under 35 U.S.C. §103

The examiner has rejected claims 3-8, 10-12, 25, 27, and 29 over Huyghe *et al.* in combination with a variety of secondary references. However, as stated above, Huyghe *et al.* fails to anticipate the present claims as it does not address infecting cell populations at a specific point in the population growth curve. As such, each of the obviousness rejections based on Huyghe *et al.* is improper for the same reason. Applicants provide the following comments on the deficiencies of the secondary references merely to complete the record.

Kraft *et al.* As stated previously herein, the present invention does not address infection of host cells at a particular point in the ***cell cycle***. Rather, the terms "mid-log" and "stationary" phase address a point in the host cell population growth curve. Thus, to the extent Kraft may discuss infection of cells during S phase in synchronized cultures with an adenovirus, it does not address the present invention.

Garnier *et al.* or Perrin *et al.* The Perrin reference relates to a rabies virus system quite distinct from adenovirus. The fact that Perrin teaches perfusion in the context of rabies virus production would in no way motivate the use of perfusion in the context of adenovirus production. The reason for this is that rabies virus is an enveloped "budding" RNA-based

rhabdovirus whereas adenovirus is a DNA capsid based non-enveloped virus of an entirely different viral family – these viruses infect and grow differently and indeed replicate differently. Moreover, adenovirus is a very fragile virus as compared to viruses like rabies virus and one would not expect that it could be handled in any where near the same manner as rabies virus. Therefore, there would be no *a priori* expectations that the optimal conditions in one system would be optimal or even functional in another system.

More importantly, Perrin says nothing about advantages in terms of ease of purification and purity that one might obtain through the use of a low rather than high perfusion rate. This in itself is strong evidence of non-obviousness, and a finding that is in no way taught or suggested by Perrin.

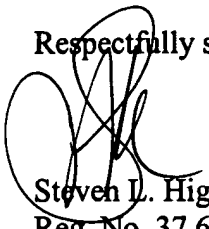
Furthermore, it appears as though the perfusion rate disclosed by Perrin is a fairly high rate in that it involves an exchange of 144% of the reactor volume/day (page 1245, col. 2). From the description provided, it would appear that this 144% exchange would correspond to maintenance of a glucose level at significantly greater than 2 g/L based on the present inventors' experience in the preferred systems which they employ that a 100% volume exchange per day will maintain a glucose level of approximately 1.5 g/L.

The Garnier reference does not appear to teach or suggest that low perfusion rates, characterized as rates that will maintain a glucose level of less the 2 g/L, will provide particular advantages in the production of highly purified adenovirus. Indeed, Garnier relates to improved recombinant protein production and says nothing about the effects of fed batch on adenovirus particle production and says nothing about whether fed batch would have an advantageous effect on the ability to provide highly purified adenoviral particles.

VI. Conclusion

In light of the foregoing, applicants respectfully submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. The examiner is invited to contact the undersigned attorney at (512) 536-3184 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



Steven L. Highalander
Reg. No. 37,642
Attorney for Applicant

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: February 13, 2002

APPENDIX A: MARKED UP COPY OF CLAIMS

3. (Amended) The process of claim 1, wherein the producer cells are [seeded using an] essentially homogeneous [pool of cells] with respect to cell the phase of growth.

18. (Amended) The process of claim 1, wherein said recombinant adenovirus is a replication-deficient adenovirus encoding a [therapeutic] selected gene operably linked to a promoter.

23. (Amended) The process of claim [1] 18, wherein said [recombinant] selected gene is selected from the group consisting of antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl* antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, mda-7, thymidine kinase or p53.

24. (Amended) The process of claim 23, wherein said [recombinant] selected gene is a p53 gene.

25. (Amended) The process of claim [1] 18, wherein said promoter is an SV40 IE, RSV LTR, β-actin, CMV-IE, adenovirus major late, polyoma F9-1, or tyrosinase promoter.

APPENDIX B: CLEAN COPY OF CLAIMS (UNOFFICIAL)

1. A process for preparing recombinant adenovirus, the process comprising:

   (a) preparing a culture of producer cells in a selected media;

   (b) infecting producer cells in the culture with recombinant adenovirus, wherein the producer cells are infected between mid-log phase of growth and stationary phase of growth; and

   (c) harvesting recombinant adenovirus from the cell culture.

2. The process of claim 1, wherein the producer cells are infected with the adenovirus between late-log phase and stationary phase of growth.

3. The process of claim 1, wherein the producer cells are essentially homogeneous with respect to cell the phase of growth.

4. The process of claim 1, wherein the producer cells are perfused for at least a portion of the time that the cells are cultured.

5. The process of claim 4, wherein the producer cells are perfused at a rate that will maintain a glucose level of between about 0.5 and about 3.0 gm glucose/liter.

6. The process of claim 5, wherein the producer cells are perfused at a rate that will maintain a glucose level of between about 0.7 and about 2.0 gm glucose/liter.

7. The process of claim 6, wherein the producer cells are perfused at a rate that maintains a glucose level of between about 1 and about 1.5 gm glucose/liter.

8. The process of claim 1, wherein the producer cells are seeded into the culture medium and allowed to attach to a culture surface for between about 3 hours and about 24 hours prior to infection with adenovirus.

9. The process of claim 1, wherein the culture medium is at least partially recirculated during the adenovirus infection step.

10. The process of claim 1, wherein the culture medium is seeded with between about 0.5 x 10^4 and about 3 x 10^4 cells/cm2.

11. The process of claim 10, wherein the culture medium is seeded with between about 7.5 x 10^3 and about 2.0 x 10^4 cell/cm2.

12. The process of claim 11, wherein the culture medium is seeded with between about 9 x 10^3 and 1.5 x 10^4 cells/cm2.

13. The process of claim 1, wherein the harvested adenovirus is subjected to purification and placed into a pharmaceutically acceptable composition.

14. The process of claim 13, the adenovirus is purified by steps which include chromatography.

15. The process of claim 14, wherein the chromatography step involves subjecting the adenovirus to more than one chromatographic separations.

16. The process of claim 14, wherein the chromatography step involves subjecting the adenovirus to only one chromatographic separation.

17. The process of claim 16, wherein the chromatographic separation includes ion-exchange chromatography.

18. The process of claim 1, wherein said recombinant adenovirus is a replication-deficient adenovirus encoding a selected gene operably linked to a promoter.

19. The process of claim 18, wherein said replication deficient adenovirus is lacking at least a portion of the E1 region.

20. The process of claim 19, wherein said producer cells complement the growth of replication deficient adenovirus.

21. The process of claim 1, wherein said producer cells are selected from the group consisting of 293, PER.C6, 911 and IT293SF cells.

22. The process of claim 21, wherein said producer cells are 293 cells.

23. The process of claim 18, wherein said selected gene is selected from the group consisting of antisense *ras*, antisense *myc,* antisense *raf,* antisense *erb,* antisense *src,* antisense *fms,* antisense *jun,* antisense *trk,* antisense *ret,* antisense *gsp,* antisense *hst,* antisense *bcl* antisense *abl,* Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, mda-7, thymidine kinase or p53.

24. The process of claim 23, wherein said selected gene is a p53 gene.

25. The process of claim 18, wherein said promoter is an SV40 IE, RSV LTR, β-actin, CMV-IE, adenovirus major late, polyoma F9-1, or tyrosinase promoter.

26. The process of claim 1, wherein the adenovirus is harvested by steps that include lysing the producer cells by means other than freeze-thaw.

27. The process of claim 26, wherein the producer cells are lysed by means of a detergent lysis.

28. The process of claim 26, wherein the producer cells are lysed by means of autolysis.

29. The process of claim 1, further comprising purifying the harvested adenovirus to obtain a purified recombinant adenovirus composition having one or more of the following properties:

(a) a virus titer of between about 1×10^9 and about 1×10^{13} pfu/ml;

(b) a virus particle concentration between about 1×10^{10} and about 2×10^{13} particles/ml;

(c) a particle:pfu ratio between about 10 and about 60;

(d) having less than 50 ng BSA per 1 x 10e12 viral particles;

(e) between about 50 pg and 1 ng of contaminating human DNA per 1×10^{12} viral particles,

(f) a single HPLC elution peak consisting essentially of 97 to 99% of the area under the peak.

Biochemical Methods in Cell Culture and Virology

ROBERT J. KUCHLER
Rutgers—The State University

Dowden, Hutchinson & Ross, Inc.
STROUDSBURG, PENNSYLVANIA

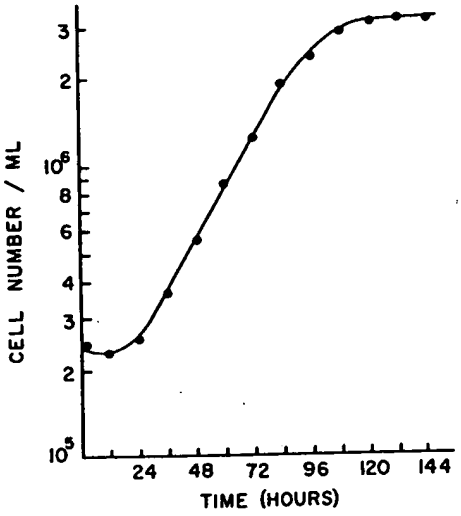


Figure 3-1
Population growth curve for L-M strain mouse fibroblasts in suspension culture.

cultured cells ranges from 12 to 48 hours. Since cultures are usually started with 50,000 to 200,000 cells, four or five population doublings occur during the culture cycle. A population of cells will stop dividing when an essential nutrient is depleted or an inhibitory substance is produced. At this point the culture enters the plateau phase, which is characterized by the fact that a constant cell number prevails over a period of time. The constancy of cell number may result because division ceases in all cells or because some cells degenerate and die while others continue to divide.

Replicate-Culture Technique

Replicate culturing is used to establish a series of identical cultures that can be harvested separately at various times to represent the entire population (Evans et al., 1951; Rinaldini, 1954). In growth studies with cells attached to surfaces, a replicate set of cultures must be used, since a single culture must be harvested for analysis at each time. Cells growing in agitated suspension cultures provide more flexibility, because aliquots can be removed from a single large culture at various times to measure the growth response. The replicate-culture technique is invaluable in quantitative physiological studies where various cultures are subjected to different experimental conditions. Three general procedures for setting up replicate cultures are described.

Manual procedure

Ordinary pipettes can be used to dispense a set of replicate cultures in 1- to 10-ml volumes. The cell pool should be continuously agitated by hand or with a magnetic stirrer.

Automatic procedure

A Cornwall pipetting unit is also useful for distributing small volumes of cell suspension. The intake bulb is immersed in the cell suspension, which is continu-

used as a standard. Ribose between 2 and 20 μg can also be used. Selection of an accurate standard is difficult because the purine-to-pyrimidine ratio of the RNA to be analyzed may not be known.

Protein by the Lowry reaction

The quantitative determination of protein is made by reacting the Folin-Ciocalteau reagent with the alkali-solubilized precipitate after the extraction of DNA and RNA. The blue color that develops is a result of (1) biuret reaction of proteins with copper ion in alkali, and (2) reduction of phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophan present in the treated protein. Since a cell contains between 300 and 600 pg of protein, the reaction can be carried out using 0.5 to 1.0×10^5 cells.

Reagents

Reagent A: dissolve 0.5 g of $CuSO_4 \cdot 5H_2O$ and 1 g of sodium or potassium tartrate in 100.0 ml of water.

Reagent B: carbonate-copper solution. Mix 50.0 ml of 2 percent Na_2CO_3 with 1.0 ml of reagent A. Discard after 1 day.

Reagent C: alkaline copper solution. Mix 50.0 ml of 2 percent Na_2CO_3 in 0.1 *N* NaOH with 1.0 ml of reagent A. Discard after 1 day.

Reagent D: diluted Folin reagent. Dilute the Folin–Ciochalteau reagent to make it 1 *N* in acid.

Reaction. Add up to 0.2 ml of sample containing 5 to 100 μg of protein and 1.0 ml of reagent B to a test tube. Mix well and allow to stand for 10 minutes or longer at room temperature. Add 0.1 ml of reagent D rapidly with immediate mixing. After 30 minutes or longer, the color is read in a spectrophotometer. For 5 to 25 μg of protein per ml, it is desirable to make the readings at or near the absorption maximum at 750 nm. For more concentrated solutions the readings may be made at 500 nm. Reagent C is used instead of reagent B when proteins are encountered that are difficult to solubilize.

Standard. Bovine serum albumin ranging from 5 to 50 μg is used as the standard protein.

SYNCHRONOUS GROWTH

The sequential order in which biosynthetic events occur during the cell-division cycle can be ideally studied by following the activities in a single cell by cytochemical, autoradiographic, and spectrophotometric methods. Since the same cell cannot be used to follow the events throughout, populations are synchronized, so that representative samples can be removed from the culture at various points in the division cycle. During the past 20 years, some very successful methods have been developed for synchronizing populations of mammalian cells in culture, which, in turn, have led investigators to attempt to map the order of various events occurring during the cell-division cycle. One of the first studies showed that DNA is synthesized in mid-interphase, and is separated by two gaps occurring before and after mitosis (Firket and Verly, 1958). Howard and Pelc (1953) had previously recognized four stage in their studies with bean roots, and named them mitosis (M), first

gap (G1), DNA synthesis (S), and second gap (G2). In mammalian cells, the S stage most often occupies about 7 hours of the division cycle, regardless of the generation time; the M stage occupies 3 to 4 percent of the division cycle. It appears that mammalian cells have different generation times because they vary with respect to the time spent in the G1 stage (Sisken and Kinosita, 1961).

Two general procedures are employed to obtain synchronously growing populations of mammalian cells *in vitro*: (1) a small fraction of the cells in a population can be selectively isolated at a certain point in the division cycle, or the undesired cells can be preferentially destroyed; (2) all the cells, or at least a large fraction, can be blocked at a specific point in the division cycle by using an inhibitory compound, or by withholding an essential nutrient. Procedures representing both approaches are outlined.

Selective Isolation of Synchronously Growing Cells

Collection of loosely attached mitotic cells

Terasima and Tolmach (1963) introduced a simple procedure for the selective isolation of dividing cells; they exploited the observation that cells growing attached to a surface round up during the mitotic period and can be dislodged by using a gentle shearing force. The detached cells are pelleted and resuspended in a complete medium in which they grow in synchrony for one division cycle. A limitation of this method is that only about 4 percent of the cells are in the mitotic stage when the population is growing at an exponential rate, and only about one fourth of these can be obtained. The following procedure can be used to collect dividing cells.

1. Between 1 and 2×10^6 cells in 10.0 ml of a complete medium are cultured in a 100-mm plastic petri dish at 37°C in an atmosphere of 5 percent CO_2 in air.
2. The medium is discarded after 18 hours and replaced with fresh prewarmed medium of the same composition. This step is carried out to remove any dead cells that have become detached from the surface.
3. After 6 hours, the medium is again removed and discarded, and 5.0 ml of fresh prewarmed medium is forcibly ejected from a 10.0-ml pipette to wash off the loosely attached dividing cells. The force necessary to release the cells must be determined empirically.
4. The suspension of detached cells is pelleted and resuspended in a fresh or a conditioned medium. Since only small numbers of cells are obtained, the suspension is usually replicated into Leighton tubes containing cover slips.

Separation of uniformly sized cells by gravity

Synchronous cells have been separated by centrifuging exponentially growing populations in linear 2 to 10 percent (w/v) sucrose gradients made up in a complete growth medium (Sinclair and Bishop, 1965). Shall and McClelland (1971) found that cultured animal cells would also stratify according to size in a complete medium under the natural force of gravity. In the latter procedure, the cells selected for their size uniformity had a doubling time of 22 hours, whereas these